Natural Science, 2015, 7, 535-547
Published Online November 2015 in SciRes. http://www.scirp.org/journal/ns
http://dx.doi.org/10.4236/ns.2015.712054

Lipid Profile and Antioxidant Activity of Macadamia Nuts (*Macadamia integrifolia*) Cultivated in Venezuela

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Received 19 July 2015; accepted 20 November 2015; published 24 November 2015

Abstract

Macadamia nuts (*Macadamia integrifolia*) grown in Venezuela have showed an average total fat content of 70%. Oleic acid (18:1) was the main monounsaturated fatty acid (MUFA) (51.3%), followed by palmitoleic acid (16:1, 22.6%). The content of polyunsaturated fatty acids (PUFAs), C18:2 and C18:3 represented 5.4%. Thus, MUFAs and PUFAs together constituted more than 80% of the total fatty acids present. *Trans*-vaccenic acid was also present (3%). As regards to other phytochemical compounds, tocopherols and tocotrienols were not found in the sample, but the presence of squalene was detected. The antioxidant activity (44.2%) of the extract was produced by the phytochemicals present.

Keywords

Lipids, Antioxidant Activity, Fatty Acids, Macadamia Nut, Phytochemical Compounds

1. Introduction

Nuts, seeds and dried fruits have natural functional properties which can be used to develop nutritional or active foods [1]. In addition, nuts give a feeling of satiety, whilst providing high amounts of energy, minerals and vitamins that are of great interest in the development of special diets [2].

The consumption of nuts, either as seeds or oils, has been associated with a decrease in cardiovascular diseases, diabetes and obesity [3]. Nuts (walnuts, macadamia nuts, almonds, pistachios, etc.) are composed mainly of

How to cite this paper: Rengel, A., Pérez, E., Piombo, G., Ricci, J., Servent, A., Tapia, M.S., Gibert, O. and Montet, D. (2015) Lipid Profile and Antioxidant Activity of Macadamia Nuts (*Macadamia integrifolia*) Cultivated in Venezuela. *Natural Science*, **7**, 535-547. http://dx.doi.org/10.4236/ns.2015.712054

monounsaturated (40% - 60%) and saturated (7%) fatty acids, fiber, potassium, magnesium, copper, vitamin E and arginine [4]. Because of this, the regular addition of walnuts, macadamia nuts, almonds and peanuts as part of a healthy diet can result in a significant reduction of cholesterol levels in the blood. Recently the FDA has recognized dried fruits and nuts as "heart healthy" foods [1].

Macadamia nuts (**Figure 1**), which is considered to be one on the highest ranking nuts in the world for their high content and quality of their oil, are native from Australia. Australia is also the main commercial grower, producing around 40,000 tons of Macadamia nuts per year. Macadamia nuts are also grown commercially in some South American countries, such as Brazil, Costa Rica and Bolivia, as well as in Hawaii, New Zealand and Africa, with a total world annual production of 100,000 tons [5]. Minor producers of these nuts can also be found in: Bolivia, China, El Salvador, Fiji, Philippines, Indonesia, Mexico, New Zealand, Paraguay, Peru, Thailand, Tanzania, Venezuela, Zimbabwe, Rhodesia and Colombia. In Venezuela, most of the current production is in Lara state, (8.5 - 9 tons/year), where the macadamia trees are used to provide shade in coffee plantations [6].

The content of fat in these nuts represents more than 70% of their weight, however their sodium and saturated fat levels are low. Macadamia nut oil contains the highest percentage of unsaturated fats varying from 80% to 81.8% [7], compared to other oils, such as olive oil (74%) [2] and other nuts (51.6% - 67.7%) [8]. Macadamia nuts contain the highest amounts of monounsaturated fatty acids (MUFAs), predominantly oleic (60%) and palmitoleic (20%) acids of any known food [7]. Diets that contain foods with high content in MUFAs will reduce the plasma low density lipoprotein (LDL), and cholesterol levels, thus decreasing the risk of cardiovascular disease [9]. This cardio protective effect is associated with the high percentage of unsaturated fatty acids present [9].

Macadamia nuts are also rich in essential trace elements such as: calcium, iron, phosphorus, magnesium and potassium, and vitamins such as thiamine (B1), riboflavin (B2), retinol (A1), and niacin (B3).

In addition to the nutritional characteristics of macadamia nuts, some authors have reported antioxidant properties conferred by the presence of tocopherols, tocotrienols, and squalene [10]. Antioxidants are a group of molecules recognized for their ability to neutralize free radicals, and are thus considered an alternative for combating deficiencies associated with oxidative stress, such as cardiovascular and rheumatic diseases and even aging.

Squalene, which has been shown to decrease oxygen levels and prevent lipid peroxidation [10], is abundant in olive oil and quinoa and has also been found in small quantities (185 µg/g) in macadamia nuts [10].

The aim of this study was to quantify total fat and lipid profile, identify and quantify specific phytochemicals (tocopherols, tocotrienols, and squalene) and determine the antioxidant capacity of macadamia nuts cultivated in Venezuela.

2. Materials and Methods

2.1. Materials

Dried macadamia nuts were obtained from the Production and Agricultural Extension Unit "Las Lomas", Villanueva village, Hilario Luna and Luna parish, Moran municipality, Lara state, Venezuela. A total of six kg of nuts were harvested between April and November 2012. The collected nuts were stored at a temperature of 4˚C until analysis.

Figure 1. Nut of *Macadamia integrifolia*.

2.2. Methods

2.2.1. Determination of Crude Fat Content and Extraction of Lipids

Total lipid content (extracted in duplicate) was determined using the Soxhlet method [11]. The resulting oil was stored at −20˚C for the subsequent determination of tocopherols, tocotrienols and squalene.

2.2.2. Analysis of Identity Parameters of Macadamia Nut Oil

Specific gravity of was measured by using a pycnometer. Three 10-mL Fisher pycnometers, and a water bath at constant-temperature with a Micrc-set thermoregulator were used in the procedures for the specific gravity determination in, accordance with ASTM D 369 and 445 ASTM [12], respectively. The pycnometers were filled with the macadamia oil sample at a 2˚C ± 1˚C temperature below the bath temperature. It was assured to prevent direct contact between the bath fluid and the capillary opening of the pycnometer neck. This assembly was secured to the bath cover and clip and immersed inside the bath, the procedures in the ASTM standard test methods D 891 (test Method B) and D 369 [12] were followed for the density determinations and the following formula was used:

$$\text{Specific gravity} = \frac{\left(\text{Weight pycnometer} + \text{sample}\right) - \text{Weight of pycnometer}}{\left(\text{Weight pycnometer} + \text{water}\right) - \text{Weight of pycnometer}} \quad [12]$$

The determination of the refractive index (at 20˚C ± 0.1˚C) was done putting a few drops of macadamia oil with a pipette on the lower prism Abbe refractometer at 20˚C, following the procedures of Cocks and Van Rede [13].

The determination of the iodine value is based on the addition of iodine to the double bonds of unsaturated fatty acids. The result is given as g I_2 consumed by 100 g sample and is a measure for the unsaturation of oil. To determine the index of iodine: iodine index was calculated from the fatty acid composition, according to Standard AOCS [14] method Cd 1c-85. The result was expressed in g of iodine/100 grams oil. The equation used was as follows:

Iodine value = (% palmitoleic acid × 0.95) + (% of oleic acid × 0.86) + (% linoleic acid × 1732) + (% linolenic acid × 2616).

Saponification value is expressed by potassium hydroxide in mg required to saponify one (1) gram of fat. Measurement of saponification index was performed by using the formula [15]:

Saponification index = 56,000/average PM + 12.67

Where: PM average = average of molecular weight of fatty acids.

2.2.3. Determination of the Fatty acid Profile by Gas Chromatography (GC)

The fraction of lipids evaluated was obtained following the French ISO Norm 5509 (1977) [16], which describe a method for the preparation and analysis of fatty acids methyl esters from fat bodies of animal and plant origin.

The preparation of the methyl esters was realized as follows: 2 to 3 drops of the fat sample and 2 or 3 pieces of 5 mm of pumice and 3 ml of 0.2 N sodium methylate were placed in a round-bottom flask (10 cm diameter). The round-bottom flask was set up with a saponification cane to be heated under reflux for 10 minutes, with 3 mL of hydrochloric acid in 2% of methanol, which was added by the top of the cane, up to observing the discoloration of the phenolphthalein. Then sample is again heated under reflux for 10 minutes. After cooling the sample at room temperature; 8 ml hexane, 10 ml of distilled water were added. Subsequently, the assessments through gas chromatography were compared to a standard, which allowed to displaying the type and quantity of fatty acid in sample.

Gas chromatography of the fatty acids methyl esters (FAMEs), was performed according to ISO standard [17] on a HP 6890 GC System with autosampler. The column was a highly polar SGE Capillary BPX 70 of 60 m length, 0.22 mm internal diameter with 70% Cyanopropyl (equiv.) polysilphenylene-siloxane phase. Gradient oven temperature program was from 160˚C to 190˚C, heating rate: 2.5˚C/min; carrier gas: helium, flow rate 0.6 mL/min; Temperature of split-splitless injector 240˚C; Flame ionization detector (FID); flame gas: H2; software: HP Chemstation v. 3.11; sample injection: 1 µL in iso-octane.

FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards of FAMEs obtained from Sigma Chemical Co. All quantifications were done by a built-in data-handling program provided by the manufacturer of the gas chromatograph. The FA composition was reported as a relative

percentage of the total peak area.

2.2.4. Determination of the Lipid Profile by Thin Layer (TLC) Chromatography

Thin layer chromatography was carried out according to the technique described by Tuckey and Stevenson [18]. The extracted lipid fraction was applied 4 times in small drops to the upper part of a 0.25 mm silica gel plate. TLC was performed by placing the inverted plate in a tank containing hexane/diethyl ether/acetic acid (75/25/1, v/v/v). When the solvent front was 1 cm below the upper edge of the plate (approximately 10 min), plate was removed, sprayed with copper acetate saturated in water/H_3PO_4 (50/50 v/v) and then dried in an oven at 180˚C for 10 min. The components were then carbonized and identified by comparison of their migration distance on the plate, and compared with reference molecules.

Chromatography was performed with 1, 2, and 5 µL of sample, to observe the distribution of mono, di and triglycerides and fatty acids within the lipid profile.

2.2.5. Determination of Tocopherols and Tocotrienols by High Performance Liquid Chromatography (HPLC)

The content of tocopherols and tocotrienols was evaluated by HPLC following the methodology described in the international standard ISO-FDIS 9936 [19]. Samples were diluted in solvent before injection into the HPLC column as follows.

The oil used to do the determination, was obtained by using the Soxhlet's method. 2 g of dehydrated and crushed macadamia nut were weighted, and placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent (hexane) is added to the flask, and the set up is heated under reflux, when a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath. After extraction the solvent is removed, by means of a rotary evaporator, yielding the extracted compound (test sample). The non-soluble portion of the extracted solid remains in the thimble, and is discarded.

Briefly, 2.0 ± 0.1 g of the test sample was weighed and placed in a 25 mL vial added with X mL hexane and the solution were stirred to dissolve the residue. Once dissolved, the vial was filled with the same solvent. Posteriorly, 20 µL of the test solution was injected into the column and the tocopherols were identified by comparison with the chromatogram of a standard solution. The tocopherols were then quantified using the calibration factors determined from the standard solutions.

2.2.6. Determination of Squalene

Squalene was detected by thin layer chromatography using silica gel 60G. The eluents used were: 50% of chloroform/methanol/water 60/30/51 (v/v/v) and 50% of hexane/diethyl ether/formic acid at 80/20/1 ratio (v/v/v). The plates were sprayed with phosphomolybdic acid at 1.3%/H_2SO_4 10% in 100 mL ethanol. The plates were then dried in an oven at 180˚C for 10 min. The components were carbonized and identified by comparing the migration distance along the plate with standard squalene values.

2.2.7. Determination of Antioxidant Activity

The antioxidant capacity of the sample was determined as the radical trapping capacity using the free radical technique 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), following the methodology proposed by Brand-Williams *et al.* [20]: 2.9 mL of DPPH (4% concentration) was mixed with 0.1 mL of the sample at different concentrations, and spectrophotometric measurements were made at 515 nm. A standard sample was prepared by diluting 1 g of ground macadamia nut in 10 mL of methanol. The concentration ratios tested based on this solution were: 1:4, 1:5, 1:6, 1:7 and 1:8.

3. Results and Discussion

3.1. Total Crude Fat Content

The analyzed nuts contained an average of 70% oil (dry matter), which is similar to other nuts such as pine nuts (68% - 75%), hazelnuts (70% - 72%), walnuts (63% - 70%) [21]-[23] and Brazil nuts (67%) [24]. Similar results can be found in the literature for macadamia nuts of other origins. Kaijser *et al.* [25] reported between 69% - 78% of oil content in four cultivars of macadamia in New Zealand, while Maguire *et al.* [10] reported 59% of oil in

fresh macadamia nuts from Ireland. Koaze *et al.* [26], reported a range between 60% and 69.4% of fat from dehydrated nuts collected between April and August in Nairobi and central Kenya, noting that this increased towards the end of the harvest season. Nuts in our study were collected between the months of April and November 2012.

3.2. Chemical and Physical Characteristics of Crude Macadamia Nut Oil

Table 1 is compiling the chemical and physical characteristics of the macadamia oil, determined on the freshly collected nut, and compared with several vegetable oils. As can be seen in **Table 1**, the specific gravity found at the macadamia oil of 0.9213 g/ml is slightly different from those found by Carvajal and Bedoya [27] of 0.9080 g/mL, for oil from Macadamia nut of specie *tetraphylla*, and is also different to those values of other vegetable oils. The specific gravity is an important criterion of identity for edible oils, the value reported in this research, could be considered for the oil from *Macadamia integrifolia* nut.

In this study the oil from *Macadamia integrifolia* nuts showed a refractive index of 1.4679, which bears some similarity to the results of Winston and Shaw [28] for *M. intregrifolia* that varies from 1.4657 to 1.4681, and Carvajal and Bedoya [27] of 1.4607 for *M. tetraphylla*.

The average iodine index for macadamia nut oil, of the study, was determined 61.4 cg/g. Based on the classification of oils, by this standard, this oil is a non-drying oil (below 100 Iodine Index) (**Table 1**), which predicts a low percentage of polyunsaturated fatty acids, which is confirmed by its composition obtained by GC-MS. In the above classification there are some oils such as; olive oil, castor and almond oils. The value here found for *M. integrifolia* is lower than those shown by the oil of peanut oil and cashew nut.

The saponification index found for *M. intergrifolia* oil showed a mean value of 214.1 mg KOH/g; this value differed from that reported for *M. tetraphylla* oil 197.3 mg KOH/g, which is similar to those reported for walnut oil (*Juglans Regia*) of 189 - 193 mg KOH/g [28]-[30] (**Table 1**). The determination of this parameter showed that the oil of *M. integrifolia* species had a high proportion of short-chain fatty acids, characteristic property of oils like olive oil (184 - 196 mg KOH/g); as well as oils from other oilseeds such as sesame, sunflower and peanuts (**Table 1**).

The characterization of the oil was carried out immediately after the oil extraction process. It was why the evaluation of the indices of peroxide and acid were omitted. These indices may indicate deterioration by oxidative degradation or an hydrolysis by lipases respectively during the extraction process.

Unlike other similar studies, in this work was studied and summarized the identity of the oil of nut of macadamia, not only by considering type, quality and importance of its lipids, but evaluating its functional

Table 1. Analysis of identity profile of *Macadamia integrifolia* oil, compared to different vegetables oil.

Oil type	Specific gravity (g/mL)	Refractive index, η20 D	Saponification Index (mg KOH/g)	Iodine index (g/100g)
Coconut[1]	0.917 - 0.919 d	1.4480 - 1.4500 b	250 - 264 f	7.5 - 10.5 a
Sesame seed[1]	0.916 - 0.921 d	1.4700 - 1.4740 bc	188 - 195 bcd	103 - 188 e
Peanut[1]	0.909 - 0.915 bcd	1.4670 - 1.4700 bc	195 - 205 d	80 - 100 bcd
Sunflower[1]	0.915 - 0.918 cd	1.4710 - 1.4750 bc	248 - 254 f	125 - 136 de
Palm[1]	0.900 - 0.913 ab	1.4990 - 1.4520 c	176 - 182 a	-
Cahsew[2]	0.8984 a	1.4721 bc	181.8 ab	71.4 b
Olive[3]	0.910 - 0.916 bcd	1.4677 - 1.4705 bc	182 - 193 abc	80 bc
Corn[4]	0.914 - 0.921 cd	1.4701 - 1.4710 bc	187 - 193 abcd	115 - 124 cde
Soy[4]	0.915 - 0.925 d	1.4704 - 1.4744 bc	187 - 197 bcd	125 - 140 e
Walnut[5]	-	0.6500 - 0.6750 a	193–196 cd	140 - 150 e
Macadamia nut[6]	0.9080 abc	1.4607 - 1.4681 bc	-	197.3 f
Macadamia nut[7]	0.9213 d	1.4679 bc	214.1 e	61.38 b

[1]Jurado and Muñoz (2009) [50]. [2]Mujica *et al.*, (2010) [51]. [3]Codexstan (1989) [52]. [4]Bailey (1951) [53]. [5]Weston and Fryer (1920) [29]. [6]Carvajal and Bedolla, (2010) (*M. tetraphylla*) [27], and Wiston and Shaw (1943) (*M. integrifolia*) [28]. [7]Data of this researching (*M. integrifolia).* Different letters in the same column, indicate statistically significant differences ($p \leq 0.05$).

characteristics, but also the presence of tocopherols and tocotrienols (*Vitamin E*), its antioxidant capacity, and revealing the presence of squalene. Moreover, the significance and novelty of this study lies, as can be seen in **Table 1** which is comparing data of the analysis of identity for *M. integrifolia* oil from Venezuela to those found in Colombia (27) and Hawaii (28), that they are showing lightly differences among them, which could be also observed in its fatty acid profile. These differences can be attributed to environmental differences, and it is relevant to characterize the *M. Integrifolia* from different localities, before it will be used.

3.3. Determination of the Fatty Acid Profile by GC

Table 2 and **Figure 2** show the fatty acid profile of macadamia nut oils. It is important to highlight the high content of monounsaturated fatty acids (MUFAs) representing 77.5% of total fat content (**Table 3**) with 51.3% palmitoleic acid and 22.6% oleic acid. The oil contained 4.8% polyunsaturated (PUFA) including 2.4% linoleic acid and 2.4% α-linolenic acid (**Table 3**).

Oleic acid (cis 18:1 n-9) is present in animal fats and vegetable oils and has been shown to protect against cardiovascular diseases, since it reduces the levels of total cholesterol and low density lipoproteins. Both oleic acid and polyphenols, presents in olive oil, have been shown to increase high-density lipoprotein (HDL) cholesterol, and to protect HDL from oxidation, a phenomenon associated with a low cholesterol efflux from cells [31].

Table 2. Fatty acid profile of *Macadamia integrifolia* oil determined by CG.

Fatty acids	Percentage of total fats
C 12:0 Lauric	0.1
C 16:0 Palmitic	**9.4**
C 16:1 Palmitoleic (n-7)	**22.6**
C 17:0 Margaric	0.3
C 17:0 Iso Margaric	0.1
C 18:0 Stearic	**3.0**
C 18:1 (n-9) Oleic	**51.3**
C 18:1 (n-7) Trans-vaccenic	**3.0**
C 18:2 (n-6) Linoleic	**2.4**
C 20:0 Arachidic	**2.5**
C 18:3 (n-3) α-linolenic	**2.4**
C 20:1 (n-9)	0.3
C 22:0 Behenic	0.8
C 22:1 (n-9) Erucic	0.3
C 24:0 Arachidonic	0.3
Other	1.2
Total	100

Table 3. Saturation profile of fatty acids in *Macadamia integrifolia* oil.

Fatty acid type	Percentage of total fats
Saturated fatty acids	16.5
Monounsaturated fatty acids (MUFAs)	77.5
Polyunsaturated fatty acids (PUFAs)	4.8
Others	1.2
Total	100

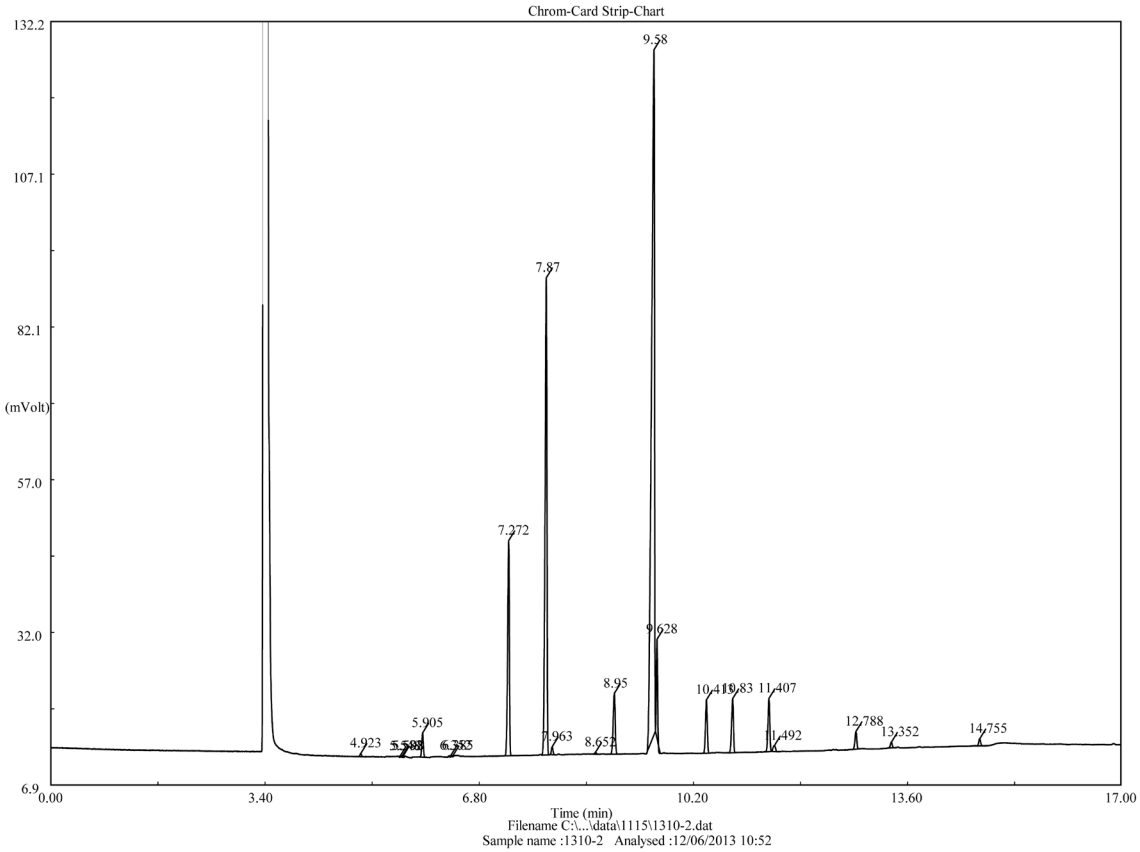


Figure 2. Detection of fatty acid profile of *Macadamia integrifolia* oil determined by CG.

Polyunsaturated fatty acids (PUFAs) are classified in function of the position of their last double bond at the methyl end of the molecule. According to this, n-3 and n-6 PUFAs (2.4%, respectively) were are present in Macadamia oil (**Table 2**). The majority of fatty acids can be synthesized by mammals from the carbohydrates in the diet, but two of these: linoleic acid (LA, 18:2 n-6) and alpha-linolenic acid (LNA, 18:3 n-3) cannot be synthesized endogenously. These are nonetheless necessary as precursors of the long-chain PUFAs and for the proper functioning of the organism [32]. Both ω-6 and ω-3 polyunsaturated fatty acids help to prevent diseases of the circulatory system, as they significantly reduce the levels of total cholesterol and low-density lipoproteins in plasma [33].

The sum of MUFAs and PUFAs represented more than 80% of the total fatty acids in the sample (**Table 3**). It is worth noting the presence of *trans*-vaccenic acid (C 18:1 n-7; 3% of total fatty acids) in macadamia nuts cultivated in Venezuela. Vaccenic acid (from the Latin *vacca* (cow)) is an omega-7, naturally occurring trans-fatty acid found in the milk of ruminants and milk products and has also been found in small concentrations in human milk [34] [35]. Its IUPAC name is 11-octadecenoic, and its lipid shorthand name is 18:1 trans-11 [36]. Trans fatty acids are generally considered to have a negative effect on human health, vaccenic acid is not harmful; on the contrary some authors found apparently positive effects. Lock *et al.* [37] indicated that a number of components of milk fat have anti-carcinogenic properties including conjugated linoleic acid and vaccenic acid. Moreover, *trans* vaccenic acid is of great interest due to its bioconversion to conjugated linoleic acid by Δ9 desaturase. This bioconversion, characteristic of ruminants, also occurs in the tissues of non-ruminant animals and humans [34] [38] [39]. In the macadamia nuts here assessed, only 16.5% of the total fatty acids were saturated, with palmitic acid being the most abundant (9.4%) (**Table 2**).

Palmitic acid enriches the phospholipids of cell membranes, interfering with the normal function of low density lipoprotein receptors, reducing their absorption and thus increasing their concentration in the plasma. Kaijser *et al.* [25] reported that mono-unsaturated fatty acids represent 80% of the total fats in macadamia nut oil whereas saturated fatty acids were only found in a range from 13.2% to 17%. The content of polyunsaturated

fatty acids (18:2 + 18:3) is relatively low, ranging from 2.8% to 4.7%. These data are comparable with other reports in the literature [40] [41]. Despite this, it is important to consider that the fatty acid composition may vary substantially between different cultivars [25] and among season.

Macadamia integrifolia nut oil has a similar percentage of unsaturated fatty acids to canola oil (80.7%), peanut oil (85.2%) and corn oil (82.0%), and a higher percentage than olive oil (78%), linseed oil (56%), soya oil (79%) and cottonseed oil (72.7%) [42].

The percentage of specific fatty acids in macadamia nut oil is high compared to many other oils: linolenic acid (C: 18:3): sunflower oil (0.93%), olive oil (0.60%), almond oil (0.93%), and maize oil (1.10%); oleic acid (C: 18:1): sunflower oil (21.3%), soya oil (21.1%), cottonseed oil (33.1%), maize oil (25.8%) and sesame oil (45,4%); stearic acid (C:18): sunflower oil (1.3%), canola oil (1.5%), cottonseed oil (1.9%) and maize oil (1.7%); palmitic acid (c:16): sunflower oil (6.4%), canola oil (3.75%) and sesame oil (9.1%) and palmitoleic acid (C: 16:1); canola oil (0.25%) [42].

3.4. Determination of the Glyceride Profile by TLC

Thin layer chromatography revealed the presence of triacylglycerides in high concentrations (top band) (**Figure 3**). Low concentrations of monoacylglycerides (MGs) were observed in the bottom band, higher concentrations of diacylglycerides DG1-2 and DG1-3 (middle bands) as shown by the intensity of the grey color, and finally, as expected, no unlinked fatty acids were found in the sample. It means that this oil was of good quality and that no unspecified lipase was found to be responsible of the enzymatic hydrolysis resulting in the production of DG1-3 and 1-2 [43]. No oxidation bands were observed, showing that oxidative rancidity did not occur.

3.5. Determination of Tocopherols and Tocotrienols

No tocopherols or tocotrienols were found in the sample (**Figure 4**). These results are consistent with Kornsteiner *et al.* [44] who also registered the absence of tocopherols and tocotrienols in macadamia nuts. However, other studies have indicated that they may be present in very small amounts from 0.6 to 2.8 µg/g [25] [45] [46]. The quality of the nut oil and its stability depends on handling practices during cultivation and harvesting, and the methods used in processing, packaging and storage. The type of packaging used is especially important as it can act as an effective barrier to some of the factors involved in the process of oxidation and loss of quality, such as light, oxygen and humidity [47].

3.6. Evaluation of Antioxidant Activity

The sample obtained was analyzed for 30 min and 60 min. A reduction of 44.2% of DPPH was observed (**Figure 5**), which translated a power of slowing or preventing the oxidative stress. Loor and Mino [48], found that at 30 min of analysis, macadamia nut extracts showed inhibition percentages between 45.7% and 93.9%, demonstrating that some samples may show up to a total reduction of DPPH.

3.7. Determination of Squalene

Squalene was detected in the analyzed samples (first bands of **Figure 6**), but not quantified. Squalene, is a hypo-

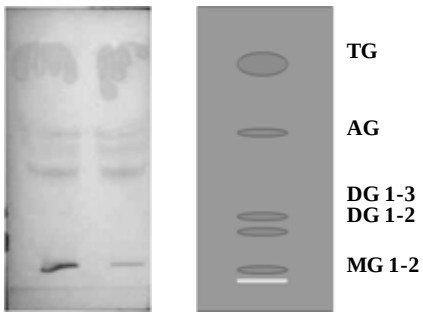


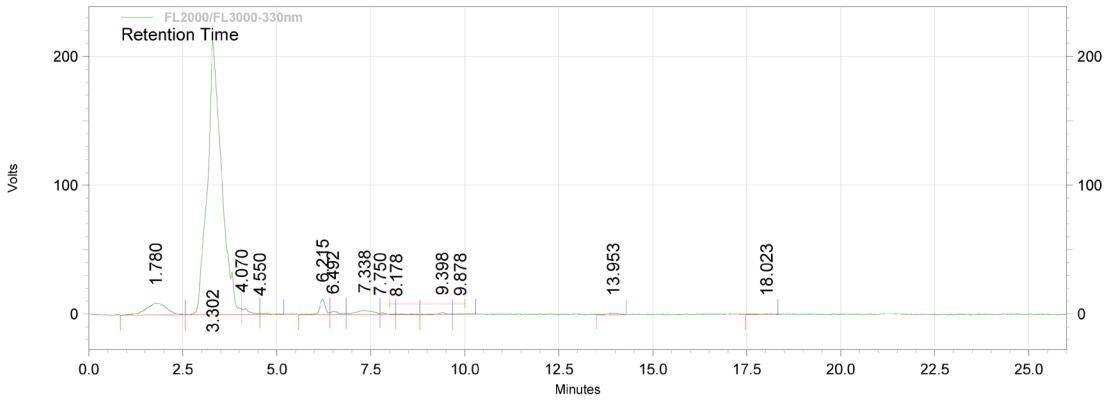
Figure 3. Lipid types in *Macadamia integrifolia* oil determined by thin layer chromatography.

Page 1 of 1 (6)

Area % Report m

Data File: C:\ChromQuest\Enterprise\Projects\Default\Data\geo\13\1310\007
Method: C:\ChromQuest\Enterprise\Projects\Default\Method\TOCOL-11-04-27.met
1 Acquired: 11/06/2013 17:44:12

eLlPrinted: 12/06/2013 15:23:00



FL2000/FL3000-330nm Results (System (12/06/2013 15:22:59) (Reprocessed))

Retention Time	Area	Pk #	Name	ESTD concentration
1.78	355220	**1**		**0.00**
3.30	5558618	**2**		**0.00**
4.07	69290	**3**		**0.00**
4.55	24002	**4**		**0.00**
6.21	127057	**5**		**0.00**
6.49	44337	**6**		**0.00**
7.34	120426	**7**		**0.00**
7.75	20953	**8**		**0.00**
8.18	11694	**9**		**0.00**
9.40	30738	**10**	**alpha**	**0.14**
9.88	12501	**11**		**0.00**
13.95	33008	**12**		**0.00**
18.02	20243	**13**		**0.00**

Figure 4. Detection of tocopherols and tocotrienols by HPLC, in *Macadamia integrifolia* oil.

cholesterolemic triterpene found in the unsaponifiable material of seed oils and animal fats. This phytochemical is a precursor of cholesterol and other sterols to which antioxidant and cardio protective properties have been attributed, since it may reduce esterified cholesterol, by the oxidation of low density lipoproteins (LDLs) in the blood [49].

4. Conclusions

The specified gravity and refractive index values found in the *Macadamia integrifolia* oil were close to those shown by *Macadamia tetraphylla*. These indices could be taken as identity criterion for the Macadamia oil. This nut oil shown also is a non-drying oil with a iodine index below 100; which is predicting a low percentage of polyunsaturated fatty acids; as is shown in the saturation profile. However the total of MUFAs and PUFAs was

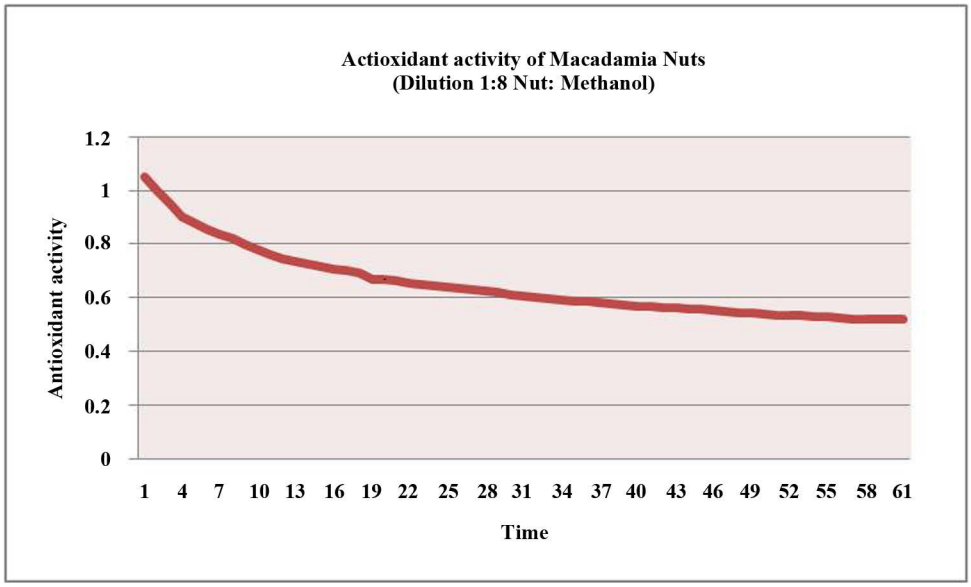


Figure 5. Antioxidant capacity of *Macadamia integrifolia* oil. (Dilution 1:8 nuts:methanol). X axis: Time, Y axis: Antioxidant capacity.

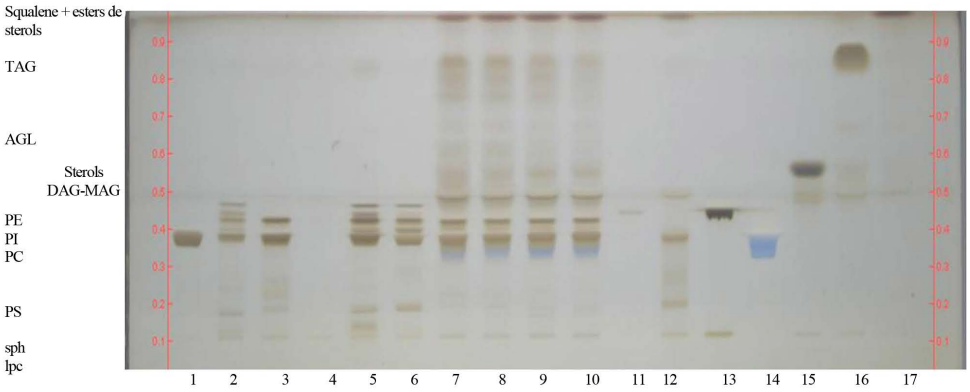


Figure 6. Detection of squalene by thin layer chromatography in *Macadamia integrifolia* oil.

above the 80% of the total fatty acids, with the oleic and palmitoleic acids, as the main MUFAs. The *Trans-vaccenic acid* was also found. Specifically we identified monounsaturated and polyunsaturated fatty acids as well as squalene molecules. We found bioactive compounds in lipids isolated from the *Macadamia integrifolia* oil from the nuts grown and processed in Venezuela. Samples were also found to show antioxidant activity, which could protect organisms from free radicals, and thus play an important role in the prevention of cancer, inflammatory activity and cardiovascular diseases. No tocopherol or tocotrienols were detected.

Acknowledgements

The authors are grateful to the National Fund for Science and Technology (FONACIT) for financing the project (PCP No. 2012000001). We are also grateful for the support given by CIRAD and we appreciate the training period of the principal author in Montpellier, France.

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